

**Stony Brook University  
The Graduate School**

Doctoral Defense Announcement

**Abstract**

“Gene recoding by synonymous mutations uncovers  
promiscuous intragenic transcription initiation in mycobacteria”

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Each genome encodes some codons more frequently than their synonyms (codon usage bias), and codons are also arranged more frequently into specific pairs (codon pair bias). Recoding viral genomes and yeast or bacterial genes with non-optimal codon pairs has been shown to decrease gene expression. Gene expression is thus importantly regulated by the use of particular codons and by their proper juxtaposition.

*Mycobacterium tuberculosis* (*Mtb*) is the causative agent of tuberculosis, one of the deadliest infectious diseases worldwide. We hypothesized that non-optimal codon pairing could be an effective strategy for attenuating gene expression to create a live vaccine for *Mtb*. We explored the role of codon pair bias by recoding essential *Mtb* genes (*rpoB*, *mmpL3*, *ndh*) and assessing their expression in the closely related and tractable model organism *M. smegmatis*.

To our surprise, recoding caused the expression of multiple smaller protein products from *rpoB*, *mmpL3* and *ndh*. These smaller proteins could not result from degradation because they expressed independently of full-length RpoB, MmpL3 or Ndh. Further investigation revealed that synonymous recoding caused transcription initiation in the middle of the open reading frame, and the smaller proteins were translated from these new transcripts. The transcripts and smaller proteins were characterized to investigate which nucleotide changes induced intragenic transcription and translation initiation. The results suggest that recombinant gene expression in mycobacteria should be carefully examined for undesired effects of codon optimization. More generally, our work expands our understanding of the codon-level parameters that control translation and transcription initiation.

**Date:** June 6, 2022

**Time:** 10:00 AM

**Place:** Laufer Lecture Hall 101

**Program:** Genetics

**Dissertation Advisor:** Jessica Seeliger